UNIVERSITI PUTRA MALAYSIA

CLINICAL PATHOLOGY AND RADIOLOGICAL ASSESMENT OF FELINE CHRONIC GINGIVOSTOMATITIS

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By

HAIDARY MOHAMMAD HUSSAIN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Veterinary Science

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DEDICATION

This thesis is dedicated to my caring, loving parents, family, friends, and my country,
May Allah reward you all with peace forever.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Veterinary Science

CLINICAL PATHOLOGY AND RADIOLOGICAL ASSESMENT OF FELINE CHRONIC GINGIVOSTOMATITIS

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December 2020

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Feline chronic gingivostomatitis (FCGS) is a severe inflammatory oral disease of cats that often refractory to treatment. The etiology is unknown but it has been attributed to bacterial agents, viral agents, and immunologic factors. The association of FCGS to dentoalveolar lesions is poorly defined. This study presents the clinical, pathological, immunofluorescence, and computed tomographic findings of 15 cats with FCGS and 7 without FCGS. Clinical examination of the oral cavity was performed and oral pathological lesions were recorded. Blood, oral swab, and excisional biopsy from palatoglossal fold were collected under general anesthesia. Histopathological analysis and immunofluorescence staining for immune cells, CD4 and CD8 were performed on the oral tissue biopsies. The swab samples were analyzed for the presence of *Bartonella henselae* and *Pasteurell*a *multocida* using PCR and bacteria culture, respectively. Blood was subjected to hematological and biochemical analysis and screened for feline calicivirus (FCV), feline herpesvirus (FHV), feline immunodeficiency virus (FIV), and feline leukemia virus (FeLV). Computed tomography (CT scan) was performed to elaborate on the dentoalveolar lesions. The main clinical findings were halitosis (73.3%), Anorexia (60%), dysphagia (53.3%), bleeding gum (33.3%), weight loss (26.7%), and ptyalism (20%). CT scan revealed different types of dentoalveolar lesions where furcation (73.3%), edentulous (66.6%), horizontal bone loss (66.6%), dental resorption (53.3%), vertical bone loss (40%), fracture (33.3%), and impacted tooth (13.3%). Viral screening was positive for FCV (86.6%), FHV (73.3%), FIV (22.2%) and FeLV (5.6%). The blood and oral swabs were negative for *Bartonella henselae.* Histopathologically, the excisional biopsies were characterized by the infiltration of inflammatory cells in the epithelium and lamina propria. It is worthy to note that, the present study reveals that, viruses like FCV, FHV, and FIV contribute greatly to the cause of FCGS and the study also discovered that, FCGS is associated with various types of dentoalveolar lesions.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains Veterinar

PATOLOGI KLINIKAL DAN PENILAIAN RADIOLOGI GINGIVOSTOMATITI KRONIK FELINE

Oleh

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Feline chronic gingivostomatitis (FCGS) adalah penyakit radang mulut yang susah untuk dirawat. Etiologi sebenar untk penyakit ini adalah tidak diketahui, walaubagaimanapun, ia telah dikaitkan dengan beberapa faktor yang melibatkan bakteria, virus dan juga imunologi. Hubungan FCGS dengan lesi dentoalveolar adalah kurang jelas. Kajian ini mengemukakan penemuan klinikal, patologi, imunofluoresensi dan tomografi daripada 22 ekor kucing yang menghidapi FCGS. Pemeriksaan klinikal rongga mulut telah dilakukan dan lesi patologi oral telah direkodkan. Sampel darah, swab dalam mulut dan biopsi dari lipatan palatoglossal telah diambil daripada kucing tersebut di bawah pengaruh anestesia umum. Analisis histopatologi dan imunofluoresensi untuk sel imun, CD4 dan CD8 telah dilakukan pada biopsi tisu rongga mulut. Sampel swab telah dianalisa untuk mengesan kehadiran *Bartonella henselae* dan *Pasteurella* spp menggunakan teknik kultur dan PCR. Sampel darah telah dianalisa untuk kajian hematologi dan biokimia, dan disaring untuk *feline calicivirus* (FCV), *feline herpesvirus* (FHV), *feline immunodeficiency virus* (FIV), dan *feline leukemia virus* (FeLV). Komputasi tomografi (imbasan CT) telah dilakukan untuk menghuraikan lesi dentoalveolar. Penemuan klinikal utama adalah halitosis (73.3%), anorexia (60%), disfagia (53.3%), gusi berdarah (33.3%), penurunan berat badan (26.7%) dan ptyalism (20%). Imbasan CT menunjukkan pelbagai jenis lesi dentoalveolar di mana *furcation* (73.3%), kehilangan gigi (66.6%), kehilangan tulang mendatar (66.6%), resorpsi gigi (53.3%), kehilangan tulang menegak (40%), patah tulang (33.3%), dan *impacted tooth* (13.3%). Pemeriksaan virus adalah positif untuk FCV (86.6%), FHV (73.3%), FIV (26.6%), dan FeLV (6.6%). Sampel darah dan swab dalam mulut didapati negatif untuk *Bartonella henselae*. Secara histopatologi, biopsi telah dicirikan dengan penyusupan sel-sel radang di *epithelium* dan *lamina propria*. Kesimpulannya, FCGS boleh dikaitkan dengan pelbagai jenis lesi dentoalveolar dan kemungkinan virus seperti FCV, FHV, FIV turut menyumbang kepada perkembangan FCGS.

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LIST OF ABBREVIATIONS

AB	Antibiotic
A:G	Albumin-globulin ratio
ALP	Alkaline phosphate
ALT	Alanine transaminase
ANALG	Analgesic
BCS	Body condition score
BRL	Below reference level
C	Canine
CBCT	Cone beam computed tomography
CEJ	Cementoenamel junction
CO_2	Carbon dioxide
CSD	Cat scratch disease
CT scan	Computed tomography
CWCC	Complete white cell count
DI	Deionized water
DLH	Domestic long hair
DM	Diabetes mellitus
DNA	Deoxyribonucleic acid
DSH	Domestic short hair
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme linked immunosorbent assay
ENZ	Enzyme
F	Female

FCGS	Feline chronic gingivostomatitis
FCV	Feline calicivirus
FeLV	Feline leukemia virus
FHV	Feline hepesvirus
FIP	Feline infectious peritonitis
FITC	Fluorescein isothiocyanate
FIV	Feline immunodeficiency virus
FORL	Feline odontoclastic resorptive lesion
FPV	Panleukopenia virus
FR	Fully recovered
G	Gram
G1	Grade 1
GGT	Gamma glutamyl transpeptidase
Hb	Haemoglobin
HE	Haematoxylin and Eosin
HIV	Human immunodeficiency virus
I	Incisor
IF	Immunofluorescence
IFA	Immunofluorescence antibody
IgA	Immunoglobulin A
IgG	Immunoglobulin G
ITS	Internal transcribed spacer
IU	International unite
Kg	Kilogram
KV	Kilovolt

L	Litre
M	Male
M	Molar
MAb	Monoclonal antibody
MALT	Mucosa associated lymphoid tissue
mAs	Milliampere per shot
MCV	Mean cell volume
MCHC	Mean cell haemoglobin concentration
Mg	Milligram
MGJ	Mucogingival junction
Mm	Millimetre
Mmol	Millimole
N	Number
NBF	Neutral buffered formalin
ND	None determine
NEG	Negative
NF	Neutered female
NM	Neutered male
NSAID	Non-steroidal anti inflammation drug
OLP	Oral lichen planus
RBC	Red blood cell
P	Premolar
PCR	Polymerase chain reaction
PCV	Pocked cell volume
PD	Periodontal disease

PE	Phycoerythrin
PG	Plaque grade
POS	Positive
PR	Partial remission
rFeIFN	Recombinant feline interferon
rFeIFN-ω	Recombinant feline interferon omega
RNA	Ribonucleic acid
SAID	Steroidal anti inflammation drug
SDAI	Simple disease activity index
SE	Standard error
SM	Supportive medicine
STM	Stuart transport medium
SX	Surgery
Th cell	T helper cell
U	Unit
UPM	Universiti Putra Malaysia
URL	Upper reference level
UV	Ultraviolet
UVH	University Veterinary Hospital
µL	Microlite

CHAPTER 1

INTRODUCTION

1.1 Background

Feline chronic gingivostomatitis (FCGS) as the name implies, is a disease condition of cats characterized by the inflammation of the oral cavity and refractory to treatments. At a certain time, the inflammation is called lymphocytic plasmacytic when it involves numerous lymphocytes and plasma cells (Hung *et al.,* 2014); or the names correspond to the site of inflammation within the oral cavity such as the pharynx (stomatitis pharyngitis) (Healey *et al*., 2007), lymphoplasmacytic gingivitis (Baird, 2005), gingivitis stomatitis (Frost et al., 1986), feline gingivitis stomatitis pharyngitis (Diehl and Rosychuk, 1993) and feline chronic gingivostomatitis (Healey *et al.,* 2007; Dolieslager *et al*., 2011; Harley *et al.,* 2011; Rolim *et al.,* 2017; Silva *et al*., 2018).

In FCGS the typical manifestations include; inflammation of areas within the oral cavity such as the gingiva, buccal mucosa, and the glossopalatine fold which is the caudal region of the oral mucosa. The inflammation is usually chronic and severe, the palate, oropharynx, and tongue are occasionally involved (Farcas *et al*., 2014). Many studies revealed there are two specific common sites for the inflammation namely; tissues at lateral to the palatoglossal fold (which causes faucitis), and below the molar and premolar arches exist a buccal mucosa (which causes buccal stomatitis) (Southerden, 2010).

The prevalence of feline chronic gingivostomatitis was reported by Healey *et al.* (2007) where 0.7 to 13% of cats examined to veterinary practices (Lund *et al.,* 1999) of cats examined to. In cats, three oral diseases were most commonly found, i.e. periodontal disease (PD), feline chronic gingivostomatitis (FCGS), and feline odontoclastic resorptive lesions (FORL). About 38.2% of cats displayed oral disease, out of which, 17.6% of cats (6 cats) showed PD, while FCGS was found in 11.8% of the cats (4 cats) with 8.8% (3 cats) showing feline FORL (Whyte *et al.,* 2017).

However, it is still not well understood the mechanism of the pathophysiology and the causative agent of FCGS, several factors such as; environmental stress (Loesche, 1986), breed predisposition, several infectious agents (Healey et al., 2007) such as virus and bacteria (Southerden and Gorrel, 2007), are all attributed to the cause of FCGS. Infectious agents such as the feline calicivirus (FCV), feline herpesvirus (FHV), feline immunodeficiency virus (FIV), and feline leukemia virus (FeLV), are also attributed to the FCGS. Although feline leukemia and feline immunodeficiency are known to infect cats and cause immunosuppression, the fundamental roles these diseases played in the development of FCGS are still unknown and debated. (Winer *et al.,* 2016; Fernandez *et al.,* 2017; Rolim *et al.,* 2017).

About 500 species of bacteria have been reported as the microbes causing oral cavity disease in cats. They are the aerobic, anaerobic, and facultative bacteria, mycoplasma, and yeast (Dolieslager *et al.,* 2013). Previous studies have documented some differences in the bacterial flora between healthy and diseased oral cavities of cats (Dolieslager *et al.,* 2011; 2013)

The clinical signs of cats with FCGS are excessive drooling, severe oral pain, dysphagia, anorexia, inappetence, ptyalism, halitosis, and weight loss (Harley *et al*., 2011). The diagnosis of FCGS is usually based on the clinical appearance and home oral hygiene measures, response to professional periodontal therapy, and tooth extraction. Biopsies are rarely necessary but are indicated if there is an uncommon presentation such as asymmetry or lesions in atypical positions (Southerden, 2010). However, the main factors responsible for the development of FCGS are mostly infectious and/or immunosuppressive conditions of the cat (Healey *et al*., 2007).

FCGS condition has been associated with an immunological response wherein the mucosal lesion, the cytokines profiles are responsible for up-regulation of type 1 and type 2 T-helper cytokines (Ahlfors et al., 1996). In a chronic case of FCGS, the typical histopathological changes within the oral cavity are inflammatory infiltrate which are characterized by lymphocytes, plasma cells, granulocytes, macrophages, and mast cells respectively, (Harley *et al*, 2011; Arzi *et al.,* 2010).

Treatment of FCGS is usually through a medical or surgical approach. FCGS is mostly refractory to medical treatment (Silva et al., 2018). A multimodal approach using oral antiseptics, non-steroidal anti-inflammatory medication, and intermittent antibiotics can be successfully used too. However, the present interest in FCGS management has shifted toward the use of interferon (Southerden, 2010).

1.2 Statement of the problem

The status of FCGS in Malaysia is still not known . There is a knowledge gap in the literature about FCGS among Malaysian cats. Considering the multifactorial causes with different immune cells implicated, there is a need to carry out this study.

1.3 Hypotheses

First hypothesis

H_0 = the clinical and pathological characteristics of the cat with FCGS is not different from that of normal healthy cats and FCGS is not associated with dentoalveolar lesions as compared to the cat without FCGS.

H_A = the clinical and pathological characteristics of the cat with FCGS are different from that of normal healthy cats and FCGS is associated with dentoalveolar lesions as compared to the cat without FCGS.

Second hypothesis

H_0 = There is no relationship between increasing immune cell population and severity of FCGS.

H_A= There is a relationship between increasing immune cell population and severity of FCGS.

1.4 Objectives

The present study has the following objectives:

1. To identify and compare the clinical and oral pathological lesion in cats with and without FCGS
2. To determine the immunofluorescence findings and histopathological changes in the mucosal biopsies of cats with and without FCGS; and
3. To characterize the severity of FCGS and compare the dentoalveolar findings in cats with or without FCGS using computed tomography scan
4. To determine the occurrence of FCGS in cats presented to the University Veterinary Hospital from 2008 to 2018.

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APPENDICES

Appendix 1

Animal Assessment and Monitoring Sheet

Case number ( ) Date: / /2019

Clinical examination			Pathological examination		
No	**Evaluation items**	**Evaluation criteria**	**No**	**Evaluation items**	**Evaluation criteria**
1	Age	( ) months	1	Number of the clinically edentulous	Incisor( ),Canine( ),Premolar( ),Molar( )
2	Sex	M( ), MN( ), F( ), FN( )	2	Plaque grade (PG)	Incisor( ),Canine( ),Premolar( ),Molar( )
3	Breed	1) Domestic short hear 2) Domestic long hear 3) Pure bred 4) Crossbred	3	Gingival inflammation	Incisor( ),Canine( ),Premolar( ),Molar( )
			4	Severity of inflammation	Incisor( ),Canine( ),Premolar( ),Molar( )
			5	Mandibular lymph node palpation	Normal ( ), Enlargement ( )
			6	Bleeding gum	Yes ( ), No ( )
4	Weight	( ) Kg	7	Halitosis (bad breath)	Yes ( ), No ( )
5	Body condition score	1( ),2( ),3( ),4( ),5( )	8	Ptyalism	Yes ( ), No ( )
6	Salivary pH	pH ( )	9	Dysphagia	Yes ( ), No ( )
7	Vaccination status	1) Fully vaccinated ( ) 2) Particular vaccinated ( ) 3) Unvaccinated ( )	10	Weight loss	Yes ( ), No ( )
			11	Anorexia	Yes ( ), No ( )
			12	Pyrexia	Yes ( ), No ( )
8	Environment	1) Indoor with other cats 2) Indoor without other cats 3) Out door	13	Change in grooming behavior	Yes ( ), No ( )
			14	Change in drinking behavior	Yes ( ), No ( )
			15	No signs	Yes ( ), No ( )

M: Male; MN: Male neutered; F: Female; FN: Female neutered; ND: Non determine; Plaque grade plaque index by Loë (1967); Severity of inflammation (Harley et al., 2011).

Appendix 2

Immunofluorescence Protocol for Tissue Sections

Note:
Do not let the tissues dry out once they are re-hydrated.
Materials:

1. Paraffin- embedded tissue slides
2. Coverslips
3. Slide racks & tray
4. Staining dishes with lids
5. Orbital shaker
6. PAP pen & Transfer pipettes
7. Deionized water (DI H_2O)
8. PBS (Phosphate Buffered Saline)
9. Conjugated Fluorescent antibody
10. Bovine Serum Albumin (BSA – for blocking)
11. Glycerol

Staining procedure (Cover staining dishes with a lid in each step)

1. Dip slides in two (2) changes of xylene for 5 min each.
2. Dip slides in one (1) change of 100% alcohol for 5 min.
3. Dip slides in one (1) change of 95% alcohol for 5 min.
4. Dip slides in one (1) change of 80% alcohol for 5 min.
5. Dip slides in one (1) change of 70% alcohol for 5 min.
6. Rinse slides twice (2x) in DI H_2O for 5 minutes.
7. Soak the slides in the Sodium Citrate buffer [41 mL of 0.1 M sodium citrate (14.7 g sodium citrate dehydrate to 500 mL DI H_2O, pH=6] and cover with a lid.
8. Microwave until the liquid boils, about 1 - 5 min.
9. Remove from heat and let it stand at room temperature for 20 min.
10. Wash three (3) times for 5 min in DI H_2O.
11. Remove the liquid (do not touch the tissue) and use a PAP pen to circle the tissue.
12. Apply enough 5% BSA with a transfer pipette to cover the tissues.
13. Incubate the slides overnight at 4°C in a humid chamber.
14. Dilute the conjugated antibody 1:200 concentration in 1% BSA/PBS diluent.
15. Remove the BSA, then incubate with conjugated antibody solution for 1 hour at room temperature in a dark place.
16. Wash slides in PBS three (3) times 5 min each on the shaker.
17. Apply 50% glycerol/PBS to the middle of the slide.
18. Apply coverslip to slide and store slides at 4°C until it is readied to view.

Appendix 3

Haematoxylin and Eosin Staining Protocol

1. Tissues samples immediately immersed in 10% formalin.
2. Paraffin wax embedded.
3. Section cut at 5 µm.
4. Submerge slides in xylene (5 min).
5. Submerge slides in 100% alcohol (5 min).
6. Submerge slides in 70% alcohol (5 min).
7. Rinse.
8. Submerge slides in hematoxylin (5 min).
9. Rinse (3-5 time).
10. Dip slides in 1% acid-alcohol (3 sec).
11. Running tap water (5 min).
12. Submerged slides in eosin (1 min).
13. Spray slides with 95% alcohol, clean, and leave to dry.
14. Mount with DPX.
15. Ready for viewing.

Appendix 4

Polymerase Chain Reaction; Steps and Reaction

DNA from the cat's blood and swab were extracted using an innuPRED DNA mini kit (Analytik Jena, Germany). The primers were used:

1. 321s as a forward primer: 5–AGA TGA TGA TCC CAA GCC TTC TGG CG–3
2. 983 as a reverse primer: 5–TGT TCT YAC AAC AAT GAT GAT G–3

PCR steps:

Step	Temperature	Time	Cycle
Initial denaturation	95˚C	5 minutes	1x
Denaturation	94°C	30 s	30x
Annealing	54°C	30 s	30x
Extension	72°C	30 s	30x
Final extension	72°C	5 minutes 30 s	1x
Hold	12°C		-

PCR Mixture:

Components	1 reaction
Red mix	12.5 µL
ddH_2O	5.5 µL
Primer (F) 20 µM	1 µL
(R) 20 µM	1 µL
DNA template	5 µL

BIODATA OF STUDENT

Mohammad Hussain Haidary was born in the Wardak province of Afghanistan on March 18th, 1989. He received his primary and secondary education at Ibni Sina High School, Kabul, Afghanistan in 2007. He began his higher education at the Faculty of Veterinary Medicine, Herat University, Afghanistan, and obtained his bachelor's degree in Veterinary Medicine in 2012. He started his Master of Veterinary Science in Small Animal Surgery at the Faculty of Veterinary Medicine, Universiti Putra Malaysia in 2018. His research interest is veterinary surgery.

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